The *ash-1*, *ash-2* and *trithorax* Genes of *Drosophila melanogaster* Are Functionally Related

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ABSTRACT

Mutations in the *ash-1* and *ash-2* genes of *Drosophila melanogaster* cause a wide variety of homeotic transformations that are similar to the transformations caused by mutations in the *trithorax* gene. Based on this similar variety of transformations, it was hypothesized that these genes are members of a functionally related set. Three genetic tests were employed here to evaluate that hypothesis. The first test was to examine interactions of *ash-1, ash-2* and *trithorax* mutations with each other. Double and triple heterozygotes of recessive lethal alleles express characteristic homeotic transformations. For example, double heterozygotes of a null allele of *ash-1* and a deletion of *trithorax* have partial transformations of their first and third legs to second legs and of their halteres to wings. The penetrance of these transformations is reduced by a duplication of the *bithorax* complex. The second test was to examine interactions with a mutation in the *female sterile (1) homeotic* gene. The penetrance of the homeotic phenotype in progeny from mutant mothers is increased by heterozygosis for alleles of *ash-1* or *ash-2* as well as for *trithorax* alleles. The third test was to examine the interaction with a mutation of the *Polycomb* gene. The extra sex combs phenotype caused by heterozygosis for a deletion of *Polycomb* is suppressed by heterozygosis for *ash-1, ash-2* or *trithorax* alleles. The fact that mutations in each of the three genes gave rise to similar results in all three tests represents substantial evidence that *ash-1, ash-2* and *trithorax* are members of a functionally related set of genes.

GENES of Drosophila which give rise to homeotic mutations can be classified according to whether they normally "function selectively in particular segments" or are "required in all segments" for the correct expression of those genes which do function selectively in particular segments (STRUHL 1983). Examples of the former class include genes of the *bithorax* complex (LEWIS 1978; BENDER *et al.* 1985; AKAM 1983; SANCHEZ-HERRERO *et al.* 1985) and the *Antennapedia* complex (DENELL *et al.* 1981; WAKIMOTO, TURNER AND KAUFMAN 1984; CARROLL *et al.* 1986; MAHAFFEY and KAUFMAN 1987; GLICKSMAN and BROWER 1988). Loss of function mutations in these genes only cause transformations in specific segments and the products of these genes are normally only expressed in those specific segments.

By contrast, mutations in the latter class of genes can cause transformations in all or nearly all of the segments. Mutations in *ash-1, ash-2* or *trithorax* can cause homeotic transformations affecting all of the imaginal discs (Table 1). The nature of most transformations caused by *ash-1, ash-2* or *trithorax* mutations is similar to transformations caused by mutations in the former class of genes which "function selectively in particular segments." So, an individual homozygous for a loss of function mutation in any one of the *ash-1, ash-2* or *trithorax* genes could be described as expressing a *proboscipedia* transformation (labial to leg/antenna), and an *aristapedia* transformation (arista to tarsus), and a *Sex combs reduced* transformation (prothorax to mesothorax), and an *Ultrabithorax* transformation (metathorax to mesothorax), etc.

The similarity in the spectrum of homeotic transformations caused by mutations in *ash-1, ash-2* and *trithorax* and evidence that double mutations in *ash-1* and *ash-2* caused an enhanced phenotype led to the hypothesis that these genes represent a functionally related set (SHEARN, HERSPERGER and HERSPERGER 1987). Three independent lines of genetic evidence are presented here to support this hypothesis. The rationale of the genetic tests used is that if the products of these three genes are involved in the same cellular function then loss of function mutations in *ash-1* and/or *ash-2* should show similar interactions with mutations in other genes as have already been reported for loss of function mutations in *trithorax*.

MATERIALS AND METHODS

Mutant stocks: The *ash-1* (3-49; 76B-D) and *ash-2* (3-76; 96A) genes were originally identified in a screen for third chromosome, late larval/early pupal lethals which cause imaginal disc defects (SHEARN *et al.* 1971). The symbol *ash* is an acronym for the kinds of imaginal disc defects caused by different alleles of these two genes: discs **a**bsent, **s**mall, or **h**omeotic. All of the *ash-1* and *ash-2* alleles used in this study have been previously described (SHEARN, HERSPERGER and HERSPERGER 1987). The *trithorax* (*trx*, 3-54; 88B) gene is also on the third chromosome. It was originally identified by a mutation called *Regulator of bithorax* which was isolated by E. B. LEWIS. The deletion of *trithorax* used in this study $Df(3R)red^{P93}$, was also isolated by E. B. LEWIS. It deletes 88A10-88C2-3. The EMS induced allele, trx^{E3}, was isolated by KENNISON and TAMKUN (1988) as a dominant suppressor of the extra sex combs phenotype of a *Polycomb* mutation. *Df(3R)P9* is a deletion of the entire *bithorax* complex (BX-C); *Dp(3:1)P115* is the duplication segregant of the transposition *Tp(3:1)P115* which includes the entire BX-C (LEWIS 1978). The maternal-effect lethal mutation, $fs(1)h^1$, was isolated by GANS *et al.* (1975) as a temperature-sensitive, female-sterile mutation. Its homeotic phenotype was described by FORQUIGNON (1981). The *fs(1)h* gene (1-21) is uncovered by $Df(1)sn^{c128}$ (LEFEVRE and JOHNSON 1973) which deletes 7D1-7D5-6. The deletion of *Polycomb* used, *Df(3L)Asc* (78D1-2; 79A4-C1), was isolated by G. JÜRGENS and described by HAYNIE (1983) and CAPDEVILA, BOTAS and GARCIA-BELLIDO (1986). For a description of the mutations and balancer chromosomes used see LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1985, 1986, 1987).

Interaction crosses: All cultures were maintained at 20° in 10-dram shell vials on a medium of cornmeal, autolyzed yeast, molasses, and agar with Tegosept added as a mold inhibitor. Each vial was seeded with a suspension of live yeast. All crosses were done at 20° except those with *Df(3L)Asc*, which were done at 27° in order to maximize the penetrance of the extra sex combs phenotype. As pointed out by KENNISON and TAMKUN (1988) and as observed in these studies the penetrance and expressivity of the transformations caused by the mutations studied is sensitive to growth conditions. To minimize this source of variability, a standard procedure was adopted for all crosses described here. Five females and five males were placed in a vial and transferred every 24 h for 4–10 days.

Interactions of *ash-1*, *ash-2* and *trithorax* mutations with each other: All of the *ash-1* and *ash-2* mutations are on chromosomes with the recessive marker mutation *red Malpighian tubules* (*red*, 3-53.6). The deletion of *trithorax*, $Df(3R)red^{P93}$, is also a deficiency of the *red* gene. In crosses between flies which are heterozygous for these mutations and balancer chromosomes, the relevant double and triple heterozygous progeny can be recognized by the eye color caused by homozygosis for *red*. Comparisons of the numbers of such progeny with the numbers of their sibs heterozygous for the balancer chromosomes indicated that none of these mutations had a dramatic effect on viability even as double or triple heterozygotes (data not shown).

Effect of BX-C gene dosage on penetrance of homeotic transformations: Flies heterozygous for both $ash\text{-}1^{RF605}$ and $Df(3R)red^{P93}$ have transformations of the metathorax to mesothorax which resemble those observed in BX-C mutants. To examine whether flies heterozygous for both a deletion of BX-C and either $ash\text{-}1^{RF605}$ or $Df(3R)red^{P93}$ also expressed such transformations, $ash\text{-}1^{RF605}$ or $Df(3R)red^{P93}$ heterozygotes were mated to flies of the genotype *Df(3R)P9/Dp(3R)P5, Sb*. To examine the affect of a duplication of BX-C on the penetrance of homeotic transformations caused by heterozygosis for both $ash\text{-}1^{RF605}$ and $Df(3R)red^{P93}$, females heterozygous for $Df(3R)red^{P93}$ were mated to males of the genotype $Dp(3{:}1)P115;\ ash\text{-}1^{RF605}red/TM1$ (derived from crossing *Dp(3:1)P115; Df(3R)P115/TM1* females to $ash\text{-}1^{RF605}red/TM3$ males). The female progeny of the cross which are marked with *red* are heterozygous for both $ash\text{-}1^{RF605}$ and $Df(3R)red^{P93}$ and have three doses of BX-C; the male progeny which are marked with *red* are also heterozygous for both $ash\text{-}1^{RF605}$ and $Df(3R)red^{P93}$ but have two doses of BX-C.

Interactions with a mutation in the *fs(1)h* gene: The effect of *ash-1* and *ash-2* mutations on the penetrance of homeotic transformations caused by maternal $fs(1)h^1$ hemizygosis was compared to that previously described by DIGAN *et al.* (1986) for $Df(3R)red^{P93}$. Females hemizygous for $fs(1)h^1$ were generated by crossing females of the genotype $Df(1)sn^{c128}/Basc$ to males of the genotype $fs(1)h^1/Y$. The hemizygous females $[fs(1)h^1/Df(1)sn^{c128}]$ were mated to males with the genotype *Gl/mutant* where *mutant* stands for an allele of *ash-1*, *ash-2*, or *trithorax* or a deletion of BX-C. These males were generated by crossing *Gl/TM1* females to males heterozygous for an allele of *ash-1*, *ash-2*, or *trithorax* or a deletion of BX-C. For each cross the *Gl/+* progeny served as the control. This was necessary because, as can be seen in the control column of Table 4, even at 20° the penetrance of homeotic transformations in the progeny of mothers hemizygous for $fs(1)h^1$ varies and can be as high as 9%. For each mutation tested, the significance of the difference in penetrance between the sibling experimental and control flies was evaluated using the G-test (SOKAL and ROHLF 1969).

Interaction with a mutation in the *Polycomb* gene: Females heterozygous for a deficiency which includes the *Polycomb* gene, *Df(3L)Asc*, were mated to males heterozygous for alleles of *ash-1*, *ash-2*, or *trithorax*. Male progeny were examined for the presence of sex comb teeth on their mid and hind legs using a stereomicroscope at 30× magnification. This method of analysis provides a conservative estimate of the degree of suppression, since a leg with a single sex comb tooth bristle is scored the same as one with a complete sex comb. The *t*-test was used to evaluate the significance of the difference between the mean number of legs with sex comb teeth per male heterozygous for the deficiency of *Polycomb* alone compared to those heterozygous for that deficiency and an allele of *ash-1*, *ash-2*, or *trithorax*.

RESULTS

Phenotype of mutant alleles: A wide variety of homeotic transformations is caused by mutations in the *ash-1*, *ash-2*, or *trithorax* genes (Table 1). Transformations affecting all of the imaginal discs have been recognized (SHEARN *et al.* 1971; INGHAM and WHITTLE 1980; SHEARN 1980; INGHAM 1981, 1985; SHEARN, HERSPERGER and HERSPERGER 1987). There are only two differences in the variety of transformations caused by mutations in these genes. One difference is that none of the *ash-2* mutations so far examined express the posterior wing to anterior wing transformation. The highest penetrance observed for this transformation among *ash-1* mutations is 50% for $ash\text{-}1^{III\text{-}10}$ homozygotes (SHEARN, HERSPERGER and HERSPERGER 1987). Most other alleles of *ash-1* do not express this transformation at all. This incomplete penetrance probably indicates that many of the *ash-1* alleles that have been examined are leaky alleles. The fact that none of the alleles of *ash-2* so far studied (SHEARN, HERSPERGER and HERSPERGER, 1987; A. SHEARN, unpublished observation) express this transformation of posterior wing to anterior wing may indicate that the alleles of *ash-2* so far studied are also leaky alleles. The only other difference in the variety of transformations listed in Table 1 involves the trans-

TABLE 1

Homeotic transformations caused by mutations in the *ash-1*, *ash-2* or *trx* genes

Transformation	Gene[a]		
	ash-1	*ash-2*	*trx*
Proboscis → leg and/or antenna	$+^b$	$+^c$	$+^d$
Antenna → leg	$+^b$	$+^e$	$+^d$
Humerus → wing	$+^b$	$+^c$	$+^f$
Leg 1 → leg 2	$+^b$	$+^c$	$+^f$
Posterior wing → anterior wing	$+^b$	−	$+^g$
Haltere → wing	$+^e$	$+^c$	$+^f$
Leg 3 → leg 2	$+^b$	$+^c$	$+^f$
abd 2–7 → abd 1	−	−	$+^f$
Genitalia → leg and/or antenna	$+^e$	$+^c$	$+^d$

[a] + means that homozygous mutant alleles of this gene have been reported to cause the indicated transformation. Superscript indicates reference to the original data in footnotes.
[b] SHEARN (1980).
[c] SHEARN, HERSPERGER and HERSPERGER (1987).
[d] INGHAM (1985).
[e] SHEARN *et al.* (1971).
[f] INGHAM and WHITTLE (1980).
[g] INGHAM (1981).

TABLE 2

Penetrance of homeotic transformations in double and triple heterozygotes of *ash-1*, *ash-2* and *trx* mutations

Genotype[a]			No. flies	Percent transformation[b]	
ash-1	*trx*	*ash-2*		pro → meso	meta → meso
RF605 / +	**+** / *Df(3R)red^{P93}*	**+** / +	231	3.5	52.4
+ / *RF605*	***Df(3R)red^{P93}*** / +	**+** / +	365	39.7	27.9
+ / *III-10*	***Df(3R)red^{P93}*** / +	**+** / +	235	9.8	5.1
+ / +	***Df(3R)red^{P93}*** / +	**+** / *703*	377	15.6	0.2
+ / +	***Df(3R)red^{P93}*** / +	**+** / *1803*	344	2.9	0.3
+ / *III-10*	***Df(3R)red^{P93}*** / +	**+** / *703*	56	82.1	1.8
+ / *III-10*	***Df(3R)red^{P93}*** / +	**+** / *1803*	71	5.6	2.8

[a] Maternally derived genes are indicated in boldface.
[b] pro → meso = partial transformation of prothorax to mesothorax, *i.e.*, leg 1 to leg 2; meta → meso = partial transformation of metathorax to mesothorax, *i.e.*, leg 3 to leg 2 and/or haltere to wing.

formation of posterior abdominal segments to anterior abdominal segments. That transformation was only observed for the spontaneous, nonlethal allele, $trx^{1}1$ (INGHAM and WHITTLE 1980). Homozygous clones of lethal alleles of *trithorax* do not express such transformations in the abdomen (INGHAM 1985) nor do homozygous clones of *ash-1* or *ash-2* lethal alleles (A. SHEARN, unpublished observation).

Interactions of *ash-1*, *ash-2* and *trithorax* mutations with each other: If the similarity in the variety of homeotic transformations caused by mutations of *ash-1*, *ash-2* and *trithorax* reflects that the products of these genes are involved in the same cell function, then mutations in any one of these genes should enhance the phenotype caused by mutations in either of the other two genes. Previous studies indicated that such interactions did occur. Double heterozygotes of a weak allele of *ash-1*, ($ash\text{-}1^{III\text{-}10}$) and a deletion of *trithorax* showed a slightly enhanced phenotype (CAPDEVILA and GARCIA-BELLIDO 1981). Double homozygotes of $ash\text{-}1^{III\text{-}10}$ and either $ash\text{-}2^{703}$ or $ash\text{-}2^{1803}$ also expressed an enhanced phenotype (SHEARN, HERSPERGER and HERSPERGER 1987). The results presented in Table 2 provide additional evidence of such interactions. Flies heterozygous for either $ash\text{-}1^{RF605}$, a putative null allele, or $Df(3R)red^{P93}$, a deletion of *trithorax*, express no detectable transformations of halteres to wings or third legs to second legs (data not shown). CAPDEVILA and GARCIA-BELLIDO (1981) also found a low penetrance of such transformations (0–1%) among flies heterozygous for deletions of *trithorax*. However, among flies heterozygous for both $ash\text{-}1^{RF605}$ and $Df(3R)red^{P93}$, 52.4% or 21.8% (depending on the maternal genotype) express partial transformations of halteres to wings and/or partial transformations of third legs to second legs (Table 2). Examples of such transformations are presented in Figure 1. The haltere transformations are recognized by the presence of scutellar bristles on the metanotum and/or wing margin bristles on the capitellum. The third leg transformations are recognized by the presence of apical bristles on the distal tibia. Some double heterozygotes, 3.5% or 40.7% (depending on the maternal genotype), also express a partial transformation of first legs to second legs (prothoracic to mesothoracic). This transformation is most easily recognized by the presence, on first legs, of sternopleura and/or apical bristles which are characteristic of second legs. In males, this prothoracic to mesothoracic transformation also causes a reduced number of sex comb teeth on the basitarsus of first legs. Clearly, the degree of penetrance of the prothoracic to mesothoracic transformation compared to the metathoracic to mesothoracic transformation does not depend only on the zygotic genotype but also depends on the maternal genotype. For example, the penetrance of prothoracic to mesothoracic transformations is greater than the penetrance of metathoracic to mesothoracic transformations if $Df(3R)red^{P93}$ is maternally derived (Table 2). This is evidence that there is a maternal as well as a zygotic component to the interaction between these genes.

Double and triple heterozygotes of other alleles of

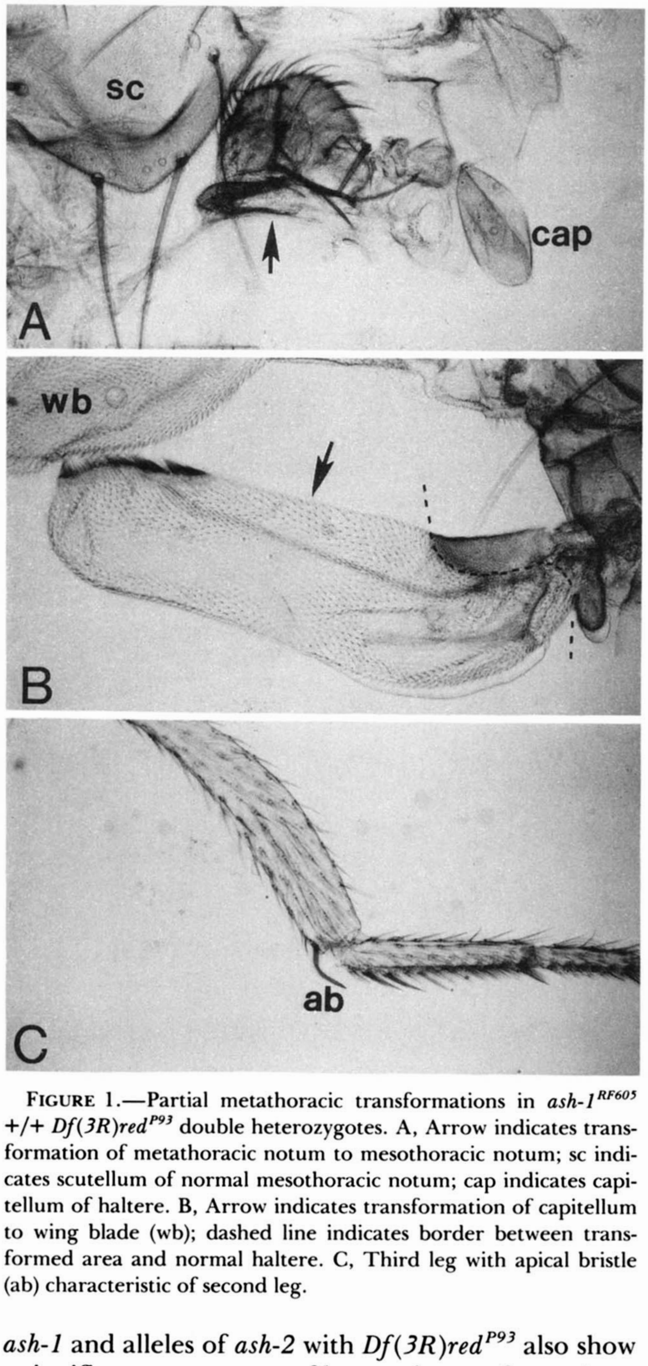

FIGURE 1.—Partial metathoracic transformations in *ash-1*^{RF605} +/+ *Df(3R)red*^{P93} double heterozygotes. A, Arrow indicates transformation of metathoracic notum to mesothoracic notum; sc indicates scutellum of normal mesothoracic notum; cap indicates capitellum of haltere. B, Arrow indicates transformation of capitellum to wing blade (wb); dashed line indicates border between transformed area and normal haltere. C, Third leg with apical bristle (ab) characteristic of second leg.

TABLE 3

Effect of BX-C dosage on penetrance of homeotic transformations in single and double heterozygotes of *ash-1*^{RF605} and *Df(3R)red*^{P93}

Genotype^a			No. flies	Percent transformation^b	
ash-1	*trx*	*BXC*		pro → meso	meta → meso
RF605 / +	**+** / +	**+** / *Df(3R)P9*	176	0.0	14.8
+ / *RF605*	**+** / +	***Df(3R)P9*** / +	140	0.0	0.0
+ / +	***Df(3R)red***^{P93} / +	**+** / *Df(3R)P9*	223	0.0	2.2
+ / *RF605*	***Df(3R)red***^{P93} / +	**+** / +	172^c	48.3	26.7
+ / *RF605*	***Df(3R)red***^{P93} / +	**+** / *Dp(3:1)P115*	143^d	11.9	12.0

^{a} Maternally derived genes are indicated in boldface.

^{b} pro → meso = partial transformation of prothorax to mesothorax, *i.e.*, leg 1 to leg 2; meta → meso = partial transformation of metathorax to mesothorax, *i.e.*, leg 3 to leg 2 and/or haltere to wing.

^{c} Males derived from the cross of +/+; *Df(3R)red*^{P93}*/Bal* females to *Dp(3:1)P115/Y; ash-1*^{RF605} *red/Bal* males.

^{d} Females derived from the cross of +/+; *Df(3R)red*^{P93}*/Bal* females to *Dp(3:1)P115/Y; ash-1*^{RF605} *red/Bal* males.

ash-1 and alleles of *ash-2* with *Df(3R)red*^{P93} also show a significant penetrance of homeotic transformations. The increase in penetrance is much less with weak alleles of *ash-1* than with *ash-1*^{RF605}. The penetrance of metathoracic to mesothoracic transformations in flies heterozygous for both *ash-1*$^{III\text{-}10}$ and *Df(3R)red*^{P93} is 5.1%. This value is essentially the same as the 7.2% reported previously by CAPDEVILA and GARCIA-BELLIDO (1981) for the same genotype. For *ash-2*^{703}, the penetrance of the prothoracic to mesothoracic transformation in flies also heterozygous for *Df(3R)red*^{P93} is low but significant ($P < 0.005$ according to the G-test). This shows that *ash-2* also interacts with *trithorax*. The penetrance of the prothoracic to mesothoracic transformation is 82.1% among heterozygotes for the double mutant, *ash-1*$^{III\text{-}10}$*ash-2*^{703} and *Df(3R)red*^{P93}. This is much higher than the sum of the penetrance with *ash-1*$^{III\text{-}10}$ alone (9.8) and *ash-2*^{703} alone (15.6).

Effect of BX-C gene dosage on the penetrance of homeotic transformations: Since *trithorax* mutations cause homeotic transformations of the haltere and third leg which are similar to those caused by mutations in the *Ultrabithorax* gene of the BX-C (LEWIS 1968; INGHAM and WHITTLE 1980), several investigators have looked for and found interactions between *trithorax* and *Ubx* mutations (INGHAM 1980; CAPDEVILA and GARCIA-BELLIDO 1981; CAPDEVILA, BOTAS and GARCIA-BELLIDO 1986; SATO and DENELL 1987). The effect of BX-C gene dosage on the penetrance of homeotic transformations caused by heterozygosis for *ash-1*^{RF605} and/or *Df(3R)red*^{P93} is presented in Table 3. Among flies heterozygous for *ash-1*^{RF605} and *Df(3R)P9*, which deletes the entire BX-C, 14.8% express transformations from metathorax to mesothorax, but only if the *ash-1*^{RF605} mutation is derived from the mother. If the *ash-1*^{RF605} mutation is derived from the father the penetrance is zero, which indicates that this interaction depends upon an *ash-1* maternal-effect. Among flies heterozygous for *Df(3R)red*^{P93} and *Df(3R)P9* only 2.2% express transformations from metathorax to mesothorax if the *Df(3R)red*^{P93} mutation is derived from the mother. A similar low penetrance was observed by CAPDEVILA and GARCIA-BELLIDO (1981) for flies deficient for both *trithorax* and BX-C when the *trithorax* deficiency was derived from the mother. The penetrance of metathorax to mesothorax transformations in male flies with two doses of the BX-C but heterozygous for maternally derived $Df(3R)red^{P93}$ and paternally-derived $ash\text{-}1^{RF605}$ is 26.7%, a value that is much higher than the penetrance in flies heterozygous for either maternally derived $Df(3R)red^{P93}$ (2.2%) or paternally derived $ash\text{-}1^{RF605}$ (0%) and hemizygous for BX-C. Moreover, 48.3% of these males heterozygous for maternally derived $Df(3R)red^{P93}$ and paternally derived $ash\text{-}1^{RF605}$ also express a partial transformation of first leg to second leg (pro → meso). The penetrance of both transformations is significantly reduced ($P < 0.005$ according to G-test) among sibling females heterozygous for maternally derived $Df(3R)red^{P93}$ and paternally derived $ash\text{-}1^{RF605}$ but which also have a duplication, *i.e.*, three doses, of the entire BX-C. The difference in sex between these two classes of progeny is not primarily responsible for the difference in penetrance. This conclusion is based on analyzing the penetrance of these transformations among sibling males and females which were heterozygous for maternally derived $Df(3R)red^{P93}$ and paternally derived $ash\text{-}1^{RF605}$ and which had two doses of the BX-C (Table 2). Among 216 progeny, the penetrance of prothoracic to mesothoracic transformations was 40.7% and the penetrance of metathoracic to mesothoracic transformations was 21.8%. Neither of these values are significantly different ($P > 0.05$ according to G-test) than those for the males of that identical genotype reported in Table 3 (48.3% and 26.7%, respectively).

Interactions with a mutation in the *fs(1)h* gene: The *fs(1)h* gene was originally identified by a recessive, temperature-sensitive, *X* chromosome mutation (originally called *1456* and now called $fs(1)h^1$) which at a restrictive temperature is both a maternal-effect lethal and a pupal lethal (GANS, AUDIT and MASSON 1975). It was discovered subsequently that progeny, derived from homozygous $fs(1)h^1$ mothers in which oogenesis occurred at an intermediate temperature (23°), exhibited a substantial frequency of missing halteres and/or third legs and a low frequency of homeotic transformations of the haltere to wing and third leg to second leg (FORQUIGNON 1981). If the progeny of homozygous $fs(1)h^1$ mothers were also heterozygous for a deletion of *trithorax* the frequency of metathoracic to mesothoracic homeotic transformations was markedly increased (FORQUIGNON 1981). The consequence of the interaction of the $fs(1)h^1$ maternal-effect and the *trithorax* zygotic effect can be interpreted either as an enhancement of the $fs(1)h^1$ maternal-effect resulting in an increased frequency of progeny expressing homeotic transformations or as an enhancement of the recessive *trithorax* mutation causing *trithorax* to act as a semidominant mutation. As a criterion for showing that *ash-1* and *ash-2* mutations behave like *trithorax* mutations, alleles of *ash-1* and *ash-2* and double mutants of *ash-1* and *ash-2* have been tested for interactions with the $fs(1)h^1$ mutation. For comparison, a deletion of BX-C and an EMS-induced allele of *trithorax* were also tested. The penetrance of metathoracic homeotic transformations in $Df(3R)red^{P93}$ heterozygotes derived from $fs(1)h^1$ hemizygous mothers is just as great as in $Df(3R)P9$ heterozygotes derived from $fs(1)h^1$ hemizygous mothers (Table 4). DIGAN *et al.* (1986) also observed a high penetrance (43%) among $Df(3R)red^{P93}$ heterozygotes derived from $fs(1)h^1$ hemizygous mothers. Heterozygosis for an EMS-induced allele (trx^{E5}) increases the penetrance but to a lesser extent (Table 4). The penetrance of these transformations in progeny derived from $fs(1)h^1$ hemizygous mothers is much higher than in progeny derived from $fs(1)h^1$ homozygous mothers. FORQUIGNON (1981) observed that the penetrance of homeotic transformations among $Df(3R)red^{P93}$ heterozygotes derived from homozygous $fs(1)h^1$ mothers was 13% and among Ubx^{130} heterozygotes it was 10%.

Heterozygosis for $ash\text{-}1^{RF605}$ increases the penetrance of homeotic transformations in progeny derived from $fs(1)h^1$ hemizygous mothers to nearly the same extent as does heterozygosis for a deletion of BX-C or *trithorax* (Table 4). Heterozygosis for other *ash-1* alleles ($ash\text{-}1^{\gamma TN402}$ and $ash\text{-}1^{III\text{-}10}$) also increases the penetrance of homeotic transformations in progeny derived from $fs(1)h^1$ hemizygous mothers, although to significantly lower levels ($P < 0.005$ according to the G-test) than does $ash\text{-}1^{RF605}$ (Table 4). Based on the phenotype of homozygous larvae, $ash\text{-}1^{\gamma TN402}$ is considered a less extreme loss of function mutation than $ash\text{-}1^{RF605}$ and $ash\text{-}1^{III\text{-}10}$ is considered a less extreme loss of function mutation than $ash\text{-}1^{\gamma TN402}$ (SHEARN, HERSPERGER and HERSPERGER 1987). So, the increase in penetrance appears proportional to the loss of *ash-1* function.

Of the two *ash-2* mutations that were tested as heterozygotes, only one, $ash\text{-}2^{1803}$, causes a small but significant increase in the penetrance of homeotic transformations in progeny derived from $fs(1)h^1$ hemizygous mothers (Table 4). Heterozygosis for the double mutant $ash\text{-}1^{III\text{-}10}ash\text{-}2^{1803}$ causes a net increase in penetrance (experimental-control) of 38.8%. The sum of the net penetrance caused by $ash\text{-}1^{III\text{-}10}$ (24.0%) and the net penetrance caused by $ash\text{-}2^{1803}$ (6.3%) is 30.3%. So, the sum of the net penetrance caused by each mutation alone is less than the net penetrance caused by the double mutant. The net penetrance caused by heterozygosis for $ash\text{-}1^{III\text{-}10}ash\text{-}2^{1803}$ (38.8%) is less than that caused by heterozygosis for $ash\text{-}1^{RF605}$ (52.2%). Homozygosis for $ash\text{-}1^{III\text{-}10}ash\text{-}2^{1803}$ causes a larval lethal phenotype indistinguishable from that

TABLE 4

Penetrance of metathoracic to mesothoracic transformations in mutant heterozygotes derived from mothers hemizygous for *fs(1)h[1]*

Mutation		Experimental [+/mutation][a]		Control [+/Gl]		Significance[b]
Gene	Allele	No. flies	Percent transformed	No. flies	Percent transformed	[G-value]
BX-C	*DF(3R)P9*	92	58.7	142	0.7	116.0***
trx	*E5*	399	48.1	216	8.8	108.0***
	Df(3R)red[P93]	511	58.5	526	1.7	472.0***
ash-1	*III-10*	281	25.3	240	1.3	72.7***
	γTN402	853	35.2	392	0.0	262.0***
	RF605	327	58.4	97	6.2	93.6***
ash-2	*703*	226	0.9	161	0.6	0.086
	1803	414	11.1	417	4.8	10.7***
ash-1 and *ash-2*	*III-10* *703*	240	27.1	273	2.2	70.1***
	III-10 *1803*	205	45.9	210	7.1	82.9***

[a] +/mutation indicates heterozygous for the mutant allele(s) in the mutation column.
[b] *** Indicates a probability of <0.005 (according to the *G*-test) that the difference between experimental and control is due to chance.

caused by homozygosis for *ash-1[RF605]* (SHEARN, HERSPERGER and HERSPERGER 1987) which was interpreted as the null phenotype.

Interaction with a mutation in the *Polycomb* gene: The dominant extra sex combs phenotype observed in adult males heterozygous for *Polycomb* mutations is sensitive to the gene dosage of *trithorax*. The extra sex combs phenotype of $Pc^3/+$ is suppressed by heterozygosis for a deletion of *trithorax* and enhanced by heterozygosis for a duplication of *trithorax* (CAPDEVILA and GARCIA-BELLIDO 1981). As shown in Table 5, *ash-1* and *ash-2* mutations also suppress this phenotype. Control males, heterozygous for a deletion of the *Polycomb* locus, *Df(3L)Asc*, express an extreme extra sex combs phenotype when raised at 27°. The mean number of legs with sex comb teeth/male fly was 5.8. Most of the males examined had sex comb teeth on all six legs and none had sex comb teeth on less than five legs. Normal males only have sex comb teeth on two legs, the prothoracic (or first) pair of legs. In males which are heterozygous for *Df(3L)Asc* and also heterozygous for *ash-1[RF605]* this phenotype is almost completely suppressed. The average number of legs with sex comb teeth is reduced to 2.4, *i.e.*, close to normal (Table 5). For comparison, in males heterozygous for *Df(3L)Asc* and also heterozygous for *Df(3R)red[P93]*, the mean number of legs with sex comb teeth is 2.1 (Table 5). An allele of *ash-2*, also significantly suppresses the extra sex combs phenotype but to a lesser extent than does either *ash-1[RF605]* or *Df(3R)red[P93]*. Males heterozygous for both *Df(3L)Asc* and *ash-2[1803]* have an average of 4.7 legs with sex comb teeth.

TABLE 5

Suppression by mutant heterozygotes of the extra sex combs phenotype caused by a deletion of the *Polycomb* gene

Genotype[a]					No. of legs with sex comb teeth	Significance[b]
Pc	*ash-1*	*trx*	*ash-2*	No. of males	Mean ± SD	*t*-value
Df(3L)Asc / +	**+** / +	**+** / +	**+** / +	115	5.8 ± 0.4	—
Df(3L)Asc / +	**+** / *RF605*	**+** / +	**+** / +	111	2.4 ± 0.6	71.8****
Df(3L)Asc / +	**+** / +	**+** / *Df(3R)red[P93]*	**+** / +	88	2.1 ± 0.4	99.2****
Df(3L)Asc / +	**+** / +	**+** / +	**+** / *1803*	110	4.7 ± 1.0	16.0****

[a] Maternally derived chromosomes are in boldface.
[b] **** indicates a probability less than 0.001 that the difference between the mean of the experimental and the mean of the unsuppressed control (5.8) is due to chance.

DISCUSSION

The *ash-1*, *ash-2* and *trithorax* genes are functionally related: Mutations in *ash-1* and *trithorax* cause similar homeotic transformations. Three lines of genetic evidence have been presented here which imply that the products of these genes are functionally related. One, recessive null alleles of these genes as double heterozygotes show a substantial penetrance of homeotic transformations whereas as single heterozygotes they show no transformations. Two, heterozygosis for null mutations in *ash-1* or *trithorax* increases the penetrance of the maternal-effect homeotic phenotype caused by $fs(1)h^1$. Three, heterozygosis for null mutations in *ash-1* or *trithorax* suppresses the extra sex combs phenotype caused by heterozygosis for a deletion of the *Polycomb* locus. The *ash-1* and *trithorax* genes appear to be part of a functionally related set that has been called the *trithorax* set (SHEARN, HERSPERGER and HERSPERGER 1987). The results presented here define the properties expected for mutations in other genes which belong to this set.

Mutations of *ash-2* express a similar variety of homeotic transformations as leaky alleles of *ash-1* or *trithorax* (Table 1). The evidence that *ash-2* is a gene which belongs to the *trithorax* set is as follows. One, double homozygotes of $ash\text{-}2^{703}$ or $ash\text{-}2^{1803}$ and leaky alleles of *ash-1* express a strongly enhanced phenotype (SHEARN, HERSPERGER and HERSPERGER 1987). Heterozygotes of one of those alleles, $ash\text{-}2^{703}$, or of the double mutant chromosome, $ash\text{-}1^{III\text{-}10}ash\text{-}2^{703}$, and a deficiency of *trithorax* show an increased penetrance of homeotic transformations (Table 2). Two, $ash\text{-}2^{1803}$ increases the penetrance of the maternal-effect homeotic phenotype caused by $fs(1)h^1$ (Table 4). Three, $ash\text{-}2^{1803}$ partially suppresses the dominant extra sex combs phenotype caused by a heterozygosis for a deletion of the *Polycomb* locus (Table 5). Thus mutations of *ash-2* exhibit all three of the properties expected for mutations in a gene of the trithorax set. However, they do so to a lesser degree than does a null allele of *ash-1* or a deletion of *trithorax*. This may indicate that none of the *ash-2* alleles tested, including ten alleles for which no data has been presented here, are null alleles. Analysis of the phenotype of *ash-2* homozygotes and trans-heterozygotes also led to the conclusion that none of the twelve *ash-2* alleles examined are null alleles (SHEARN, HERSPERGER and HERSPERGER 1987; N. TRIPOULAS, E. HERSPERGER and A. SHEARN, unpublished observations).

Other genes of the *trithorax* set: CAPDEVILA and GARCIA-BELLIDO (1981) showed that a deficiency of *trithorax* suppresses the extra sex combs phenotype caused by a *Polycomb* mutation ($Pc^3/+$) and that a duplication of the wild-type allele of *trithorax* enhances the phenotype of $Pc^3/+$. Based on these observations, KENNISON and RUSSELL (1987) screened the autosomes for other loci with a dosage dependent effect on *Polycomb* mutations. They identified several regions of the genome, including the *trithorax* region, in which an extra wild-type copy enhances the extra sex combs phenotype of $Pc^{R1}/+$. To identify the relevant genes in such regions, KENNISON and TAMKUN (1988) screened for mutations which act as dominant suppressors of *Polycomb*. They identified 13 previously unknown genes in addition to new alleles of *trithorax* and *Sex combs reduced*. It seems quite likely that some, if not all, of these genes belong to the *trithorax* set. Mutations in some of these 13 genes have already been found to increase the penetrance of the maternal-effect homeotic phenotype caused by $fs(1)h^1$ (J. A. KENNISON, personal communication). Despite the large number of mutations recovered in the screens of KENNISON and TAMKUN, it is unlikely that all of the genes of the set have yet been identified. They did not, for example, recover any mutations in *ash-1* or *ash-2* either of which can suppress the extra sex combs phenotype, as shown by the data in Table 5. Interestingly, they did recover a mutation, called *kohtalo*, which complements the lethality of *ash-1* mutations but which is in the same cytogenetic region as *ash-1*, 76B-D (SHEARN, HERSPERGER and HERSPERGER 1987; J. A. KENNISON, personal communication). A mutation isolated by KENNISON which fails to complement the lethality of both *ash-1* and *kohtalo* is a deletion from 76B1,2 to 76D5 (A. MARTINEZ-ARIAS and M. ASHBURNER, personal communication); heterozygosis for this deletion increases the penetrance of the maternal-effect of $fs(1)h^1$ to near 100% (A. SHEARN, unpublished observation).

How could the products of the trithorax set of genes be functionally related: There are, at least, two different ways in which the products of the trithorax set of genes could be functionally related. They could function catalytically in a linear pathway like the sex-determination pathway (MCKEOWN *et al.* 1987) or they could function stoichiometrically as subunits of a multimeric protein. According to either model, mutations in any one of the genes would give rise to similar phenotypes because, ultimately, a single product is affected. It is not yet possible to exclude either model. However, if the former model were correct, one ought to be able to predict the rank order of phenotypes caused by double mutations based on the severity of the phenotypes caused by the component single mutations. This expectation is based on the idea that each mutation would reduce the level of the ultimate product to a given extent. This was not possible for *ash-1* and *ash-2* mutants (SHEARN, HERSPERGER and HERSPERGER 1987). Data presented here emphasizes this point. The single mutant $ash\text{-}2^{703}$ and the double mutant, $ash\text{-}1^{III\text{-}10}ash\text{-}2^{703}$ cause much higher penetrance of first leg to second leg transformations when heterozygous with a deletion of *trithorax* [15.6% and 82.1% respectively (Table 2)] than does the single mutant $ash\text{-}2^{1803}$ or the double mutant $ash\text{-}1^{III\text{-}10}ash\text{-}2^{1803}$ [2.9% and 5.6% respectively (Table 2)]. However, $ash\text{-}2^{1803}$ and $ash\text{-}1^{III\text{-}10}ash\text{-}2^{1803}$ increase the penetrance of the maternal-effect of $fs(1)h^1$ more than do $ash\text{-}2^{703}$ or $ash\text{-}1^{III\text{-}10}ash\text{-}2^{703}$ (Table 4). Thus in one test $ash\text{-}2^{703}$ behaves as the stronger allele, while in another test $ash\text{-}2^{1803}$ behaves as the stronger allele. This pattern slightly favors the latter model, that the products of these genes are subunits of a multimeric protein and that the different activities of this protein are differentially sensitive to changes in each subunit.

The regulation of segment specific homeotic genes by the trithorax and polycomb sets of genes: The homeotic transformations caused by mutations in any one of the trithorax set of genes is similar to those caused by loss of function mutations in genes of both the *bithorax* and *Antennapedia* complexes. This similarity implies that the trithorax set of genes regulates those segment-specific homeotic genes. Indeed, the fact, that the enhanced penetrance caused by heterozygosis for both $ash\text{-}1^{RF605}$ and $Df(3R)red^{P93}$ is reduced by an extra dose of the BX-C, implies that the metathorax to mesothorax transformation, caused by heterozygosis for both mutations, results from a loss of BX-C gene function. However, the fact that the interaction of $ash\text{-}1^{RF605}$ with $Df(3R)red^{P93}$ is much stronger than the interaction of either mutation with a deletion of the BX-C, *Df(3R)P9*, implies that the products of these genes don't regulate BX-C function independently. Moreover, it is unlikely that each gene of the trithorax set regulates those segment-specific homeotic genes independently, because there appears to be so many genes in the set. Rather, this regulation may occur via the multimeric protein which is hypothesized to be the ultimate product of the trithorax set of genes.

There is another set of functionally related genes, the polycomb set, which regulates genes of the *bithorax* and *Antennapedia* complex (STRUHL and AKAM 1985; WEDEEN, HARDING and LEVINE 1986; GLICKSMAN and BROWER 1988). The polycomb set includes at least ten genes: *extra sex combs* (STRUHL 1981); *pleiohomeotic* (GEHRING 1970; DENNELL, HUMMELS and GIRTON 1989); *Polycomb* (DENELL and FREDERICK 1983); *polycombeotic* (SHEARN, HERSPERGER and HERSPERGER 1978; M. PHILLIPS and A. SHEARN, manuscript in preparation); *Polycomblike* (DUNCAN 1982); *polyhomeotic* (DURA, BROCK and SANTAMARIA 1985); *Posterior sex combs*, *Additional sex combs*, and *Sex combs on midleg* (JURGENS 1985); and *super sex combs* (INGHAM 1984). JURGENS (1985) has estimated that this set may include 40 genes. Of particular interest is the fact that mutations in the trithorax set can suppress the phenotype of mutations in the polycomb set. INGHAM (1983) showed that homozygosis for a null allele of *trithorax* suppresses the embryonic phenotype caused by homozygosis for a null allele of *extra sex combs*. CAPDEVILA and GARCIA-BELLIDO (1981) showed that a deletion of *trithorax* suppresses the extra sex combs phenotype of a *Polycomb* mutation. Data presented here show that mutations in *ash-1* and *ash-2* also suppress the extra sex combs phenotype of a *Polycomb* mutation (Table 5). CAPDEVILA, BOTAS and GARCIA-BELLIDO (1986) hypothesized that normal segment identity requires a balance between *trithorax* and the polycomb set of genes. Now that it appears the *trithorax* gene is only one member of a set of functionally related genes, their hypothesis could be revised to state that normal segment identity requires a balance between the products of the **trithorax set of genes** and the polycomb set of genes. Molecular studies of genes of the trithorax and polycomb sets should lead to an understanding of the mechanism by which the products of these two sets of genes regulate segment specific homeotic genes.

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